(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 July 2004 (15.07.2004)

PCT

(10) International Publication Number
WO 2004/058779 A1

(51) International Patent Classification[7]: C07F 9/24, C07H 21/00

(21) International Application Number: PCT/GB2003/005544

(22) International Filing Date: 18 December 2003 (18.12.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0230095.2 24 December 2002 (24.12.2002) GB

(71) Applicant *(for all designated States except US)*: **RHODIA CONSUMER SPECIALTIES LIMITED** [GB/GB]; Oak House, Reeds Crescent, Watford, Hertfordshire WD24 4QP (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **HARRIS, Christopher, John** [GB/GB]; 45 Bearcroft Avenue, Great Meadow, Worcester WR4 0DR (GB). **JACKSON, Sheena, Lesley** [GB/GB]; 81 Tamar Drive, Castle Bromwich, Brimingham B36 0ST (GB). **WILSON, David, James** [GB/GB]; 20 Leavale Road, Stourbridge DY8 2DS (GB).

(74) Agent: **BARKER, Brettell**; 138 Hagley Road, Edgbaston, Birmingham B16 9PW (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/058779 A1

(54) Title: PROCESS FOR PREPARING PHOSPHORODIAMIDITES

(57) Abstract: A method of phosphorodiamidite production comprising the steps of reacting a phosphorus trihalide with a dialkyl amine in a polar solvent to form an intermediate compound. This intermediate compound is then subsequently reacted with an hydroxyalkyl compound and a dialkyl amine in the presence of a non-polar co-solvent. Following filtration to remove the solid by-product the two solvents form separate layers. This is advantageous as the upper, non-polar solvent, layer contains the high-purity phosphorodiamidite product.

PROCESS FOR PREPARING PHOSPHORODIAMIDITES

The present invention relates to an improved method for the production of phosphorodiamidites, phosphorodiamidites produced by way of such a method and the use of such phosphorodiamidites.

Production of phosphorodiamidites has become increasingly important in the biotechnology industry. Phosphorodiamidites are used as intermediates in the manufacture of novel anti-neoplastic agents.

To be suitable for use in such industries phosphorodiamidites must be of high purity. Such phosphorodiamidites must also contain low levels of bis-(2-cyanoethyl) phosphorodiamidite (the 'diester').

This impurity is known to be a significant by product in the synthesis of 2-cyanoethyl tetraisopropylphosphorodiamidite, a commercially important intermediate in the synthesis of oligonucleotides.

As phosphorodiamidites are very air sensitive and thermally unstable, their purification is, at present, complex and expensive. Hitherto, known processes of extraction and purification of phosphorodiamidites often involve multi-stage synthetic procedures which demand the chemical isolation of intermediate materials and require extensive purification procedures prior to the isolation of high purity phosphorodiamidite products.

The present invention aims to ameliorate the aforementioned disadvantages of phosphorodiamidite production.

Accordingly, the present invention provides a method of phosphorodiamidite production which method comprises the steps of

reacting a phosphorus trihalide with a dialkyl amine in a polar solvent to form an intermediate compound and subsequently reacting the intermediate compound with a hydroxyalkyl compound and a dialkyl amine, in the presence of a non-polar co-solvent.

Following filtration to remove the solid by-product, the two solvents form separate layers. This is advantageous as the upper, non-polar solvent, layer contains the high-purity phosphorodiamidite product. The lower, polar solvent, layer contains impure product contaminated with diester and other unwanted by-products. The upper layer is then subjected to vacuum-stripping to remove the solvent, leaving the desired product with greater than 96% purity and containing less than 1% of the diester impurity. The yield of the product can further be increased by optionally rewashing the polar solvent layer with a further quantity of non-polar solvent, to give non-polar solvent solution containing pure product, from which can then be isolated high-purity phosphorodiamidite.

Advantageously, impure product contaminated with diester and other impurities which would otherwise be unsuitable for commercial use can be extracted and purified by use of the solvent purification procedure. Phosphorodiamidite products are preferentially soluble in the non-polar co-solvent whereas the diester and other unwanted polar by-products are insoluble and remain in the polar solvent layer.

Preferably, the phosphorus trihalide is phosphorus trichloride. Alternatively, the phosphorus trihalide is phosphorus tribromide.

The dialkyl amine is preferably diisopropylamine. Alternatively the dialkyl amine may be dimethylamine, diethylamine, di-n-propylamine, di-n-butylamine, di-isobutylamine or di-tert-butylamine.

The polar solvent is preferably a nitrile compound, in particular, acetonitrile. Alternatively the polar solvent may be propionitrile or benzonitrile.

The hydroxyalkyl compound is preferably hydroxypropionitrile. Alternatively the hydroxyalkyl compound may be methanol, tert-butyl alcohol or other suitable hydroxyalkyl compounds which are known to be suitable for the manufacature of phosphorodiamidites.

The alkane co-solvent is preferably heptane or hexane. Other suitable C_5 to C_9 aliphatic hydrocarbons include pentane. Suitable alicyclic hydrocarbons include, for example, cyclohexane.

The ratio of polar solvent to non-polar solvent is suitably around 1:1.

The method according to the invention provides a phosphorodiamidite compound according to Formula I:

$$(R_2\ N)_2\text{-}P\text{-}O(CH_2)_n\text{-}CN \qquad (I)$$

wherein R is a C_1 to C_4 alkyl, hydroxyalkyl or oxyalkyl group; and n is a whole number of from 1 to 4.

The compound according to formula I is preferably 2-cyanoethyl tetraisopropyl phosphorodiamidite wherein R is isopropyl, and n = 2.

The present invention also provides the use of a compound of formula I in the synthesis of oligonucleotides.

The present invention will now be illustrated, merely by way of example, as follows:

Example 1

Manufacture of 2-cyanoethyl tetraisopropyl phosphorodiamidite using hexane co-solvent

27.5g of phosphorus trichloride at ambient temperature was added to a stirred mixture of acetonitrile (200g) and diisopropylamine (121g) over 1 hour. 200g of hexane is then added followed by 14g of hydroxypropionitrile at ambient temperature over 30 minutes. The reaction mixture is then stirred for 1 hour and is then filtered to remove the solid by-product. The upper hexane layer of the filtered reaction mixture is separated and subjected to vacuum stripping to remove the hexane solvent. This leaves 20g of 2-cyanoethyl tetraisopropylphosphorodiamidite which has a purity of 96.9% when analysed by ^{31}P-NMR. The lower acetonitrile layer is stirred with a further 200g of hexane for 2 hours. The upper hexane layer from this re-extraction contains product of 98% purity when assayed by ^{31}P-NMR. Following vacuum stripping a further 11g of high purity 2-cyanoethyl tetraisopropylphosphorodiamidite is isolated.

Example 2

Manufacture of 2-cyanoethyl tetraisopropylphosphorodiamidite using heptane co-solvent

27.5g of phosphorus trichloride was added to a stirred mixture of 200g of acetonitrile and 121g of diisopropylamine at ambient temperature. 200g of heptane was then added to this mixture followed by 14.3g of hydroxypropionitrile at ambient temperature over 30 minutes. The reaction mixture was then stirred for an hour and was then filtered to remove the solid by-product. The upper heptane layer was then separated

and subjected to vacuum stripping to remove the heptane solvent leaving 22g of 2-cyanoethyl tetraisopropylphosphorodiamidite which had a purity of 96.7% when assayed by ^{31}P-NMR.

Example 3

Purification of low purity 2-cyanoethyl tetraisopropylphosphorodiamidite

60g of low purity 2-cyanoethyl tetraisopropylphosphorodiamidite (92% purity when assayed by ^{31}P-NMR containing 1.3% diester) was added to a mixture of 200g acetonitrile and 200g of heptane after stirring for ten minutes the upper heptane layer was separated and the lower acetonitrile layer stirred with a further 200g of heptane for a further 10 minutes. The second heptane fraction was then separated and the two heptane fraction subsequently combined and subjected to vacuum stripping to remove heptane solvent. 30g of 2-cyanoethyl tetraisopropylphosphorodiamidite was obtained at a purity of 98.3% when assayed by ^{31}P-NMR. This extracted phosphorodiamidite compound contained less than 0.1% of the diester impurity.

CLAIMS

1. A method of phosphorodiamidite production which method comprises the steps of reacting a phosphorus trihalide with a dialkyl amine in a polar solvent to form an intermediate compound and subsequently reacting the intermediate copound with a hydroxyalkyl compound and a dialkyl amine, in the presence of a non-polar co-solvent.

2. A method as claimed in Claim 1 in which the phosphorus trihalide is phosphorus trichloride.

3. A method as claimed in Claim 1 in which the phosphorus trihalide is phosphorus tribromide.

4. A method according to any one of Claims 1 to 3 in which the dialkyl amine is diisopropylamine.

5. A method as claimed in any one of Claims 1 to 3 in which the dialkyl amine is selected from the group consisting of dimethylamine, diethylamine, di-n-propylamine, di-n-butylamine, di-isobutylamine or di-tert-butylamine.

6. A method as claimed in any one of the preceding claims in which the polar solvent is a nitrile compound.

7. A method as claimed in Claim 6 in which the nitrile compound is acetonitrile.

8. A method as claimed in Claim 6 in which the polar solvent is propionitrile or benzonitrile.

9. A method as claimed in any one of the preceding claims in which the hydroxyalkyl compound is hydroxypropionitrile.

10. A method as claimed in any one of Claims 1 to 8 in which the hydroxyalkyl compound is methanol or tert-butyl alcohol.

11. A method as claimed in any one of Claims 1 to 10 in which the alkane co-solvent is a C_5 to C_9 aliphatic hydrocarbon.

12. A method as claimed in any one of Claims 1 to 10 in which the alkane co-solvent is an alicyclic hydrocarbon.

13. A method according to any one of the preceding claims in which the ratio of polar solvent to non-polar solvent is 1:1.

14. A phosphorodiamidite compound produced by the method of any one of Claims 1 to 13 and having the General Formula (I):

$$(R_2\ N)_2\text{-P-O}(CH_2)_n\text{-CN} \qquad (I)$$

wherein R is a C_1 to C_4 alkyl, hydroxyalkyl or oxyalkyl group; and n is a whole number of from 1 to 4.

15. A compound according to Claim 14 which is 2-cyanoethyl tetraisopropyl phosphorodiamidite.

16. The use of a compound as claimed in Claim 14 or Claim 15 as made by the method of claim 1 in the synthesis of oligonucleotides.

17. A phosphorodiamidite compound, substantially as hereinbefore described with reference to the Examples.

18. A method of phosphorodiamidite production, substantially as hereinbefore described with reference to the Examples.

19. The use of a phosphorodiamidite compound, substantially as hereinbefore described with reference to the Examples.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 03/05544

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07F9/24 C07H21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07F C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, MEDLINE, EMBASE, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 03/106468 A (RHODIA) 24 December 2003 (2003-12-24) the whole document	1-16
P,X	WO 03/087130 A (ISIS PHARMACEUTICALS INC (US)) 23 October 2003 (2003-10-23)	14-16
P,A	claims 1,40	1-13
X	PATENT ABSTRACTS OF JAPAN vol. 012, no. 075 (C-480), 9 March 1988 (1988-03-09) & JP 62 212395 A (NIPPON ZEON CO LTD), 18 September 1987 (1987-09-18)	14-16
A	abstract	1-13

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 April 2004	10/05/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Elliott, A

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HAMAMOTO S TAKAKU H: "New Approach to the Synthesis of Deoxyribonucleoside Phosphoramidite Derivatives" CHEMISTRY LETTERS, CHEMICAL SOCIETY OF JAPAN. TOKYO, JP, vol. 8, 1986, pages 1401-1404, XP002902766 ISSN: 0366-7022	14-16
A	the whole document	1-13
X	PFLEIDERER W ET AL: "Inhibition of HIV-1 replication and activation of RNase L by phosphorothioate/ phosphodiester 2',5'-oligoadenylate derivatives" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 270, no. 11, 17 March 1995 (1995-03-17), pages 5963-5978, XP002079044 ISSN: 0021-9258	14-16
A	page 5964, column 1 page 5966, column 1	1-13
A	HOUALLA D ET AL: "PREPARATIONS ET QUELQUES PROPRIETES DE COMPOSES CONTENANT LA LIAISON PHOSPHORE TRIVALENT-AZOTE" BULLETIN DE LA SOCIETE CHIMIQUE DE FRANCE, SOCIETE FRANCAISE DE CHIMIE. PARIS, FR, 1965, pages 2368-2373, XP009028565 ISSN: 0037-8968 page 2368 page 2370	1-13
X	"FLUKA CHEMIKA, BIOCHEMIKA UND ANALYTIKA KATALOG 1997/98" 1997 , FLUKA CHEMIE AG XP002277275 page 434	14-16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 03/05544

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 17-19
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 17-19

Claims 17-19 which make reference to the description have not been searched. It would appear that subject-matter conceivably falling under the possible scope of these claims is covered by claims 1-16 anyway.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 03/05544

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 03106468 A	24-12-2003	WO 03106468 A1	24-12-2003
		US 2003236233 A1	25-12-2003
WO 03087130 A	23-10-2003	US 2003225284 A1	04-12-2003
		WO 03087130 A2	23-10-2003
JP 62212395 A	18-09-1987	NONE	